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Research Article

New species of *Diaporthe* (Diaporthaceae, Diaporthales) from *Bauhinia variegata* in China

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Abstract

Diaporthe species are known as endophytes, saprobes and pathogens infecting a wide range of plants and resulting in important crop diseases. In the present study, four strains of *Diaporthe* were obtained from diseased leaves of *Bauhinia variegata* in Guangdong Province, China. Phylogenetic analyses were conducted to identify these strains using five gene regions: internal transcribed spacer (ITS), calmodulin (*cal*), histone H3 (*his3*), translation elongation factor 1-α (*tef1*) and β-tubulin (*tub2*). The results combined with morphology revealed two new species of *Diaporthe* named *D. bauhiniicola* in *D. arecae* species complex and *D. guangzhouensis* in *D. sojae* species complex.

Key words: Diaporthales, morphology, multi-gene phylogeny, taxonomy, two new taxa

Introduction

Diaporthe (syn. *Phomopsis*) is the type genus of Diaporthaceae in Diaporthales (Hyde et al. 2014; Maharachchikumbura et al. 2016). Before the implementation of "one fungus, one name", it has been a common practice to use two names for the fungal species with pleomorphic life cycles (Taylor 2011). The genus *Diaporthe* established in 1870 predates *Phomopsis* established in 1905, thus *Diaporthe* is recommended for use (Rossman et al. 2015). More than 1200 epithets for *Diaporthe* have been listed in Index Fungorum with names often based on host association (http://www.indexfungorum.org/, accessed June 2024).

The teleomorph of *Diaporthe* is characterized by aggregated spherical ascomata with tapering necks, unitunicate, 8-spored, elongate to clavate asci, and septate or aseptate, elongated to elliptical, hyaline ascospores with larger guttules at center and smaller ones at the ends (Senanayake et al. 2018; Yang et al. 2020). The anamorph is characterized by black, ostiolate pycnidia containing cylindrical phialides often producing three types of hyaline, aseptate conidia called α-conidia, β-conidia and γ-conidia (Udayanga et al. 2012a; Dissanayake et al. 2017; Fan et al. 2018). The α-conidia and β-conidia are produced frequently, but the γ-conidia are rarely observed (Gomes et al. 2013).

Diaporthe species are associated with a wide range of plant hosts as pathogens, endophytes and saprobes of crops, forest trees and ornamentals (Farr

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et al. 2002a; Crous 2005; Udayanga et al. 2012b, 2014a, 2014b, 2015; Jiang et al. 2021; Zhu et al. 2023). As plant pathogens, *Diaporthe* species cause severe diseases, e.g., leaf spots, blights, dieback, scab, decay, stem end rots and wilt of many economically important plants including species of *Citrus* (Guarnaccia and Crous 2017), *Macadamia* (Wrona et al. 2020), *Rosa* (Caio et al. 2021), *Vaccinium* (Farr et al. 2002b), *Vitis* (Manawasinghe et al. 2019) and many more (Yang et al. 2018, 2021; Guarnaccia et al. 2020; Guo et al. 2020; Ariyawansa et al. 2021). In addition, *Diaporthe* species can live inside the healthy host tissues as endophytes (Huang et al. 2015; Dong et al. 2021). In addition, species of *Diaporthe* have been also reported as saprobes from different woody hosts (Dissanayake et al. 2020).

Species identification of *Diaporthe* has traditionally been based on host as well as morphological characters such as the size and shape of fruiting bodies and spores (Mostert et al. 2001; Santos and Phillips 2009). However, recent studies have shown that many species of *Diaporthe* are not host-specific i.e., one species may infect more than one host species (Vrandecic et al. 2011; Bai et al. 2015; Zhang et al. 2018; Huang et al. 2021; Sun et al. 2021; Cao et al. 2022). Moreover, many *Diaporthe* species that are morphologically similar have proven to be genetically distinct (van Rensburg et al. 2006; Yang et al. 2018). Phylogenetic analysis using a five-locus dataset (ITS-*tef1*-*tub2*-*cal*-*his3*) has been widely used to identify species of *Diaporthe* species (Santos et al. 2017; Marin-Felix et al. 2019; Hilário et al. 2021b; Norphanphoun et al. 2022). *Diaporthe* was clustered into 13 groups, namely *D. arecae*, *D. biconispora*, *D. carpini*, *D. decedens*, *D. eres*, *D. oncostoma*, *D. pustulata*, *D. rudis*, *D. scobina*, *D. sojae*, *D. toxica*, *D. varians* and *D. vawdreyi* species complexes and nine singletons as *D. acerina*, *D. acutispora*, *D. crataegi*, *D. multiguttulata*, *D. ocoteae*, *D. perjuncta*, *D. pseudoalnea*, *D. spartinicola* and *D. undulata* based on multilocus phylogeny (Norphanphoun et al. 2022; Hongsanan et al. 2023).

Bauhinia variegata is a flowering plant species belonging to Fabaceae. It is native to China and cultivated as an ornamental tree in subtropical and tropical climate for its scented flowers. The aim of the present study was to identify new isolates collected from diseased leaves of *Bauhinia variegata* in China following the combined approaches of morphology and phylogeny in the genus *Diaporthe*.

Materials and methods

Isolation and morphological characterization

In 2022, a plant disease investigation was conducted in Guangdong Province, China. Small and irregular leaf spots were observed on the leaves of *Bauhinia variegata*, and 14 leaves were collected for isolation. The leaves were firstly surface-sterilized for 1 min in 75% ethanol, 3 min in 1.25% sodium hypochlorite and 1 min in 75% ethanol, rinsed for 2 min in distilled water and blotted on dry sterile filter paper. Then, the discolored areas were cut into 0.5 × 0.5 cm pieces and transferred to the surface of potato dextrose agar plates (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per litre), incubated at 25 °C to obtain pure cultures. The cultures were deposited in the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/) and the specimen was deposited in the Herbarium of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn/).

The isolates were grown on PDA, MEA and SNA plates, incubated at 25 °C under a 12 h near-ultraviolet light/12 h dark cycle to induce sporulation. Colony characters and pigment production on PDA, MEA and SNA were noted for the 10-day culture. Microscopic structures of the fungi growing on medium were mounted in water and examined under an Axio Imager 2 microscope (Zeiss, Oberkochen, Germany). At least 30 measurements were made for each structure examined.

DNA extraction, amplification and sequencing

The genomic DNA was extracted from the fresh mycelium harvested from PDA plates after seven days using a cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). For initial genus confirmation, the internal transcribed spacer (ITS) region was sequenced. After confirmation of *Diaporthe* species, four additional gene regions coding for translation elongation factor 1-alpha (*tef1*), beta-tubulin (*tub2*), calmodulin (*cal*) and his-tone H3 (*his3*) were sequenced. The primer pairs and amplification conditions for each of the above-mentioned gene regions are provided in Table 1.

A PCR reaction was conducted in a 20 µL reaction volume, and the components were as follows: 1 µL DNA template (20 ng/µL), 1 µL forward 10 µM primer, 1 µL reverse 10 µM primer, 10 µL T5 Super PCR Mix (containing Taq polymerase, dNTP and Mg2+, Beijing Tisingke Biotech Co., Ltd., Beijing, China), and 7 µL sterile water. Amplifications were performed using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). All amplified PCR products were evaluated visually with 1.4% agarose gels stained with ethidium bromide and PCR positive products sent to Sangon Biotech (Shanghai) Co., Ltd., (Beijing, China) for sequencing. Strands were sequenced in both directions using PCR primers. The new sequences generated in this study, as well as the reference sequences of all isolates used in the present study, are listed in Table 2.

Phylogeny

For the phylogenetic analysis, sequences of reference *Diaporthe* species and related taxa were downloaded from NCBI GenBank based on recent publications on the genus *Diaporthe* (Norphanphoun et al. 2022) (Table 2). Downloaded sequences were aligned together with the sequences obtained in the present study using MAFFT version 7.526 (Katoh and Standley 2013) and manually corrected using Bioedit 7.0.9.0 (Hall 1999). The phylogenetic analyses of the combined gene regions were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML was conducted using PhyML v. 3.0 (Guindon et al. 2010), with

Table 1. Loci assessed in this study with used PCR primers and program.

Loci	Primers	PCR: Thermal Cycles: (Annealing Temp. in Bold)	Reference
ITS	ITS1f/ITS4	(95 °C: 30 s, **48 °C**: 30 s, 72 °C: 1 min) × 35 cycles	White et al. 1990
cal	CAL228F/CAL737R	(95 °C: 15 s, **54 °C**: 20 s, 72 °C: 1 min) × 35 cycles	Carbone and Kohn 1999
his3	CYLH3F/H3-1b	(95 °C: 30 s, **57 °C**: 30 s, 72 °C: 1 min) × 35 cycles	Crous et al. 2004; Glass and Donaldson 1995
tef1	EF1-728F/EF1-986R	(95 °C: 15 s, **54 °C**: 20 s, 72 °C: 1 min) × 35 cycles	Carbone and Kohn 1999
tub2	T1(Bt2a)/Bt2b	(95 °C: 30 s, **55 °C**: 30 s, 72 °C: 1 min) × 35 cycles	Glass and Donaldson 1995; O'Donnell and Cigelnik 1997

Table 2. Isolates and GenBank accession numbers used in the phylogenetic analyses of *Diaporthe*.

Species	Location	Host	Strain	GenBank Accession Number				
				ITS	*tef1*	*tub2*	*cal*	*his3*
Diaporthe absenteum	China	*Camellia sinensis*	LC3429*	KP267897	KP267971	KP293477	NA	KP293547
D. absenteum	China	*Camellia sinensis*	LC3564	KP267912	KP267986	KP293492	NA	KP293559
D. acaciarum	Tanzania	*Acacia tortilis*	CBS 138862*	KP004460	NA	KP004509	NA	KP004504
D. acericola	Italy	*Acer negundo*	MFLUCC 17-0956*	KY964224	KY964180	KY964074	KY964137	NA
D. aceris	Japan	*Acer* sp.	LC8112	KY491547	KY491557	KY491567	KY491575	NA
D. actinidiae	New Zealand	*Actinidia deliciosa*	ICMP 13683*	KC145886	KC145941	NA	NA	NA
D. acuta	China	*Pyrus pyrifolia*	CGMCC 3.19600*	MK626957	MK654802	MK691225	MK691124	MK726161
D. alangii	China	*Alangium kurzii*	CFCC 52556*	MH121491	MH121533	MH121573	MH121415	MH121451
D. alangii	China	*Alangium kurzii*	CFCC 52557	MH121492	MH121534	MH121574	MH121416	MH121452
D. alnea	Netherlands	*Alnus* sp.	CBS 146.46	KC343008	KC343734	KC343976	KC343250	KC343492
D. amaranthophila	Japan	*Amaranthus tricolor*	MAFF 246900	LC459575	LC459577	LC459579	LC459583	LC459581
D. ambigua	South Africa	*Pyrus communis*	CBS 114015*	KC343010	KC343736	KC343978	KC343252	KC343494
D. angelicae	Austria	*Heracleum sphondylium*	CBS 111592*	KC343027	KC343753	KC343995	KC343269	KC343511
D. anhuiensis	China	*Cunninghamia lanceolata*	CNUCC 201901*	MN219718	MN224668	MN227008	MN224549	MN224556
D. arctii	Austria	*Arctium lappa*	CBS 139280*	KJ590736	KJ590776	KJ610891	KJ612133	KJ659218
D. arecae	India	*Areca catechu*	CBS 161.64*	KC343032	KC343758	KC344000	KC343274	KC343516
D. arengae	Hong Kong	*Arenga engleri*	CBS 114979*	KC343034	KC343760	KC344002	KC343276	KC343518
D. arezzoensis	Italy	*Cytisus* sp.	MFLUCC 15-0127	MT185503	NA	NA	NA	NA
D. aseana	Thailand	Unidentified dead leaf	MFLUCC 12-0299a*	KT459414	KT459448	KT459432	KT459464	NA
D. australiana	Australia	*Macadamia*	CBS 146457	MN708222	MN696522	MN696530	NA	NA
D. bauhiniicola	**China**	***Bauhinia variegata***	**CFCC 58154***	**PP864723**	**PP938599**	**PP938603**	**PP938607**	**PP938611**
D. bauhiniicola	**China**	***Bauhinia variegata***	**GZ13B**	**PP864724**	**PP938600**	**PP938604**	**PP938608**	**PP938612**
D. batatas	USA	*Ipomoea batatas*	CBS 122.21*	KC343040	KC343766	KC344008	KC343282	KC343524
D. beilharziae	Australia	*Indigofera australis*	BRIP 54792*	JX862529	JX862535	KF170921	NA	NA
D. biconispora	China	*Citrus grandis*	ZJUD62	KJ490597	KJ490476	KJ490418	MT227578	KJ490539
D. biguttulata	China	*Citrus limon*	ZJUD47*	KJ490582	KJ490461	KJ490403	NA	KJ490524
D. brasiliensis	Brazil	*Aspidosperma* sp.	CBS 133183*	KC343042	KC343768	KC344010	KC343284	KC343526
D. caatingaensis	Brazil	*Tacinga inamoena*	CBS 141542*	KY085927	KY115603	KY115600	NA	KY115605
D. camelliae-oleiferae	China	*Camellia oleifera*	HNZZ027*	MZ509555	MZ504707	MZ504718	MZ504685	MZ504696
D. caryae	China	*Carya illinoensis*	CFCC 52563*	MH121498	MH121540	MH121580	MH121422	MH121458
D. caryae	China	*Carya illinoensis*	CFCC 52564	MH121499	MH121541	MH121581	MH121423	MH121459
D. cercidis	China	*Cercis chinensis*	CFCC 52565*	MH121500	MH121542	MH121582	MH121424	MH121460
D. cercidis	China	*Cercis chinensis*	CFCC 52566	MH121501	MH121543	MH121583	MH121425	MH121461
D. chiangraiensis	Thailand	*Bauhinia* sp.	MFLUCC 17-1669*	MF190119	MF377598	NA	NA	NA
D. chrysalidocarpi	China	*Chrysalidocarpus lutescens*	SAUCC194.35	MT822563	MT855760	MT855876	MT855646	MT855532
D. cichorii	Italy	*Cichorium intybus*	MFLUCC 17-1023*	KY964220	KY964176	KY964104	KY964133	NA
D. cinmomi	China	*Cinnamomum* sp.	CFCC 52569*	MH121504	MH121546	MH121586	NA	MH121464
D. cinmomi	China	*Cinnamomum* sp.	CFCC 52570	MH121505	MH121547	MH121587	NA	MH121465
D. citriasiana	China	*Citrus unshiu*	CGMCC 3.15224*	JQ954645	JQ954663	KC357459	KC357491	KJ490515
D. columnaris	USA	*Vaccinium vitisidaea*	AR3612*	AF439625	NA	NA	NA	NA
D. compacta	China	*Camellia sinensis*	CGMCC 3.17536*	KP267854	KP267928	KP293434	NA	KP293508
D. convolvuli	Turkey	*Convolvulus arvensis*	CBS 124654*	KC343054	KC343780	KC344022	KC343296	KC343538
D. cucurbitae	Canada	*Cucumis* sp.	DAOM 42078*	KM453210	KM453211	KP118848	NA	KM453212
D. cuppatea	South Africa	*Aspalathus linearis*	CBS 117499*	KC343057	KC343783	KC344025	KC343299	KC343541
D. cyatheae	Taiwan	*Cyathea lepifera*	YMJ 1364*	JX570889	KC465406	KC465403	KC465410	NA
D. discoidispora	China	*Citrus unshiu*	ZJUD89*	KJ490624	KJ490503	KJ490445	NA	KJ490566
D. drenthii	Australia	*Macadamia*	CBS 146453	MN708229	MN696526	MN696537	NA	NA
D. durionigena	Vietnam	*Durio zibethinus*	VTCC 930005	MN453530	MT276157	MT276159	NA	NA
D. endocitricola	China	*Citrus maxima*	ZHKUCC20-0012*	MT355682	MT409336	MT409290	MT409312	NA
D. endophytica	Brazil	*Schinus terebinthifolius*	CBS 133811*	KC343065	KC343791	KC344033	KC343307	KC343549
D. eucalyptorum	China	*Eucalyptus*	CBS 132525*	MH305525	NA	NA	NA	NA

Species	Location	Host	Strain	GenBank Accession Number				
				ITS	*tef1*	*tub2*	*cal*	*his3*
D. eugeniae	Indonesia	*Eugenia aromatica*	CBS 444.82*	KC343098	KC343824	KC344066	KC343340	KC343582
D. fraxini-angustifoliae	Australia	*Fraxinus angustifolia*	BRIP 54781*	JX862528	JX862534	KF170920	NA	NA
D. fructicola	Japan	*Passiflora edulis* × *P. edulis*	MAFF 246408*	LC342734	LC342735	LC342736	LC342738	LC342737
D. fulvicolor	China	*Pyrus pyrifolia*	CGMCC 3.19601*	MK626859	MK654806	MK691236	MK691132	MK726163
D. ganjae	USA	*Cannabis sativa*	CBS 180.91*	KC343112	KC343838	KC344080	KC343354	KC343596
D. goulteri	Australia	*Helianthus annuus*	BRIP 55657a*	KJ197290	KJ197252	KJ197270	NA	NA
D. guangdongensis	China	*Citrus maxima*	ZHKUCC20-0014*	MT355684	MT409338	MT409292	MT409314	NA
D. guangxiensis	China	*Vitis vinifera*	JZB320094*	MK335772	MK523566	MK500168	MK736727	NA
D. guangzhouensis	**China**	***Bauhinia variegat*a**	**CFCC 58151***	**PP864725**	**PP938601**	**PP938605**	**PP938609**	**PP938613**
D. guangzhouensis	**China**	***Bauhinia variegata***	**GZ13E**	**PP864726**	**PP938602**	**PP938606**	**PP938610**	**PP938614**
D. gulyae	Australia	*Helianthus annuus*	BRIP 54025*	JF431299	JN645803	KJ197271	NA	NA
D. guttulata	China	Unknown	CGMCC 3.20100	MT385950	MT424685	MT424705	MW022470	MW022491
D. helianthi	Serbia	*Helianthus annuus*	CBS 592.81*	KC343115	KC343841	KC344083	KC343357	KC343599
D. heterostemmatis	China	*Heterostemma grandiflorum*	SAUCC194.85*	MT822613	MT855925	MT855810	MT855692	MT855581
D. hongkongensis	China	*Dichroa febrífuga*	CBS 115448*	KC343119	KC343845	KC344087	KC343361	KC343603
D. hordei	Norway	*Hordeum vulgare*	CBS 481.92*	KC343120	KC343846	KC344088	KC343362	KC343604
D. huangshanensis	China	*Camellia oleifera*	CNUCC 201903*	MN219729	MN224670	MN227010	NA	MN224558
D. hubeiensis	China	*Vitis vinifera*	JZB320123	MK335809	MK523570	MK500148	MK500235	NA
D. hunanensis	China	*Camellia oleifera*	HNZZ023*	MZ509550	MZ504702	MZ504713	MZ504680	MZ504691
D. infecunda	Brazil	*Schinus* sp.	CBS 133812*	KC343126	KC343852	KC344094	KC343368	KC343610
D. infertilis	Suriname	*Camellia sinensis*	CBS 230.52*	KC343052	KC343778	KC344020	KC343294	KC343536
D. kochmanii	Australia	*Helianthus annuus*	BRIP 54033*	JF431295	JN645809	NA	NA	NA
D. kongii	Australia	*Portulaca grandifla*	BRIP 54031*	JF431301	JN645797	KJ197272	NA	NA
D. krabiensis	Thailand	marine based habitats	MFLUCC 17-2481*	MN047101	MN433215	MN431495	NA	NA
D. leucospermi	Australia	*Leucospermum* sp.	CBS 111980*	JN712460	KY435632	KY435673	KY435663	KY435653
D. limonicola	Malta	*Citrus limon*	CPC 28200*	NR_154980	MF418501	MF418582	MF418256	MF418342
D. litchiicola	Australia	*Litchi chinensis*	BRIP 54900*	JX862533	JX862539	KF170925	NA	NA
D. lithocarpi	China	*Lithocarpus glabra*	CGMCC 3.15175*	KC153104	KC153095	KF576311	KF576235	NA
D. longicolla	USA	*Glycine max*	FAU599*	KJ590728	KJ590767	KJ610883	KJ612124	KJ659188
D. longispora	Canada	*Ribes* sp.	CBS 194.36*	KC343135	KC343861	KC344103	KC343377	KC343619
D. lusitanicae	Portugal	*Foeniculum vulgare*	CBS 123212	KC343136	KC343862	KC344104	KC343378	KC343620
D. lusitanicae	Portugal	*Foeniculum vulgare*	CBS 123213*	MH863280	KC343863	KC344105	KC343379	KC343621
D. malorum	Portugal	*Malus domestica*	CAA 734*	KY435638	KY435627	KY435668	KY435658	KY435648
D. manihotia	Rwanda	*Manihot utilissima*	CBS 505.76	KC343138	KC343864	KC344106	KC343380	KC343622
D. masirevicii	Australia	*Helianthus annuus*	BRIP 57892a*	KJ197276	KJ197239	KJ197257	NA	NA
D. mayteni	Brazil	*Maytenus ilicifolia*	CBS 133185	KC343139	KC343865	KC344107	KC343381	KC343623
D. megalospora	Not stated	*Sambucus canadensis*	CBS 143.27	KC343140	KC343866	KC344108	KC343382	KC343624
D. melitensis	Malta	*Citrus limon*	CPC 27873*	MF418424	MF418503	MF418584	MF418258	MF418344
D. melonis	USA	*Cucumis melo*	CBS 507.78*	KC343142	KC343868	KC344110	KC343384	KC343626
D. melonis	Indonesia	*Glycine soja*	CBS 435.87	KC343141	KC343867	KC344109	KC343383	KC343625
D. middletonii	Australia	*Rapistrum rugostrum*	BRIP 54884e*	KJ197286	KJ197248	KJ197266	NA	NA
D. millettiae	China	*Millettia reticulata*	GUCC9167*	MK398674	MK480609	MK502089	MK502086	NA
D. minusculata	China	saprobic on decaying wood	CGMCC 3.20098*	MT385957	MT424692	MT424712	MW022475	MW022499
D. miriciae	Australia	*Helianthus annuus*	BRIP 54736j*	KJ197282	KJ197244	KJ197262	NA	NA
D. musigena	Australia	*Musa* sp.	CBS 129519*	KC343143	KC343869	KC344111	KC343385	KC343267
D. myracrodruonis	Brazil	*Astronium urundeuva*	URM 7972*	MK205289	MK213408	MK205291	MK205290	17
D. nelumbonis	Taiwan	*Nelumbo nucifera*	R. Kirschner 4114*	KT821501	NA	LC086652	NA	NA
D. neoarctii	USA	*Ambrosia trifi*	CBS 109490*	KC343145	KC343871	KC344113	KC343387	KC343629
D. neoraonikayaporum	Thailand	*Tectona grandis*	MFLUCC 14-1136*	KU712449	KU749369	KU743988	KU749356	NA
D. oculi	Japan	*Homo sapiens*	HHUF 30565*	LC373514	LC373516	LC373518	NA	NA
D. osmanthi	China	*Osmanthus fragrans*	GUCC9165*	MK398675	MK480610	MK502091	MK502087	NA

Species	Location	Host	Strain	GenBank Accession Number				
				ITS	*tef1*	*tub2*	*cal*	*his3*
D. ovalispora	China	*Citrus limon*	CGMCC 3.17256*	KJ490628	KJ490507	KJ490449	NA	KJ490570
D. oxe	Brazil	*Maytenus ilicifolia*	CBS 133186*	KC343164	KC343890	KC344132	KC343406	KC343648
D. pandanicola	Thailand	*Pandanus* sp.	MFLUCC 17-0607*	MG646974	NA	MG646930	NA	NA
D. paranensis	Brazil	*Maytenus ilicifolia*	CBS 133184*	KC343171	KC343897	KC344139	KC343413	KC343655
D. pascoei	Australia	*Persea americana*	BRIP 54847*	JX862532	JX862538	KF170924	NA	NA
D. passiflorae	South America	*Passifla edulis*	CBS 132527*	JX069860	KY435633	KY435674	KY435664	KY435654
D. passifloricola	Malaysia	*Passiflora foetida*	CBS 141329*	KX228292	NA	KX228387	NA	KX228367
D. perseae	Netherlands	*Persea gratissima*	CBS 151.73*	KC343173	KC343899	KC343141	KC343415	KC343657
D. pescicola	China	*Prunus persica*	MFLUCC 16-0105*	KU557555	KU557623	KU557579	KU557603	NA
D. phaseolorum	USA	*Phaseolus vulgaris*	AR4203*	KJ590738	KJ590739	KJ610893	KJ612135	KJ659220
D. phoenicicola	India	*Areca catechu*	CBS 161.64*	MH858400	GQ250349	JX275440	JX197432	NA
D. podocarpi-macrophylli	China	*Podocarpus macrophyllus*	CGMCC 3.18281*	KX986774	KX999167	KX999207	KX999278	KX999246
D. pseudobauhiniae	Thailand	*Bauhinia* sp.	MFLU 17-1670	MF190118	MF377599	NA	NA	NA
D. pseudobauhiniae	Thailand	*Bauhinia* sp.	MFLUCC 17-1669*	MF190119	MF377598	NA	NA	NA
D. pseudolongicolla	Serbia	*Glycine max*	PL42*	JQ697843	JQ697856	NA	NA	NA
D. pseudolongicolla	Croatia	*Glycine max*	CBS 127269	KC343155	KC343881	KC344123	KC343397	KC343639
D. pseudomangiferae	Dominican Republic	*Mangifera indica*	CBS 101339*	KC343181	KC343907	KC344149	KC343423	KC343665
D. pseudooculi	Japan	*Homo sapiens*	HHUF 30617*	NR_161019	LC373517	LC373519	NA	NA
D. pseudophoenicicola	Spain	*Phoenix dactylifera*	CBS 462.69*	KC343184	KC343910	KC344152	KC343426	KC343668
D. pseudophoenicicola	Iraq	*Mangifera indica*	CBS 176.77	KC343183	KC343909	KC344151	KC343425	KC343667
D. pterocarpicola	Thailand	*Pterocarpus indicus*	MFLUCC 10-0580a*	JQ619887	JX275403	JX275441	JX197433	NA
D. pyracanthae	Portugal	*Pyracantha coccinea*	CBS 142384*	KY435635	KY435625	KY435666	KY435656	KY435646
D. racemosae	South Africa	*Euclea racemosa*	CPC 26646*	MG600223	MG600225	MG600227	MG600219	MG600221
D. raonikayaporum	Brazil	*Spondias mombin*	CBS 133182*	KC343188	KC343914	KC344156	KC343430	KC343672
D. rhodomyrti	China	*Rhodomyrtus tomentosa*	CFCC 53101	MK432643	MK578119	MK578046	MK442965	MK442990
D. rhodomyrti	China	*Rhodomyrtus tomentosa*	CFCC 53102	MK432644	MK578120	MK578047	MK442966	MK442991
D. rosae	Thailand	*Rosa* sp.	MFLUCC 17-2658*	MG828894	NA	MG843878	MG829273	NA
D. rosiphthora	Brazil	*Rosa* sp.	COAD 2914*	MT311197	MT313693	NA	MT313691	NA
D. rossmaniae	Portugal	*Vaccinium corymbosum*	CAA762*	MK792290	MK828063	MK837914	MK883822	MK871432
D. sackstonii	Australia	*Helianthus annuus*	BRIP 54669b*	KJ197287	KJ197249	KJ197267	NA	NA
D. salinicola	Thailand	*Xylocarpus* sp.	MFLU 18-0553*	MN047098	MN077073	NA	NA	NA
D. sambucusii	China	*Sambucus williamsii*	CFCC 51986*	KY852495	KY852507	KY852511	KY852499	KY852503
D. sambucusii	China	*Sambucus williamsii*	CFCC 51987	KY852496	KY852508	KY852512	KY852500	KY852504
D. schimae	China	*Schima superba*	CFCC 53103*	MK432640	MK578116	MK578043	MK442962	MK442987
D. schimae	China	*Schima superba*	CFCC 53104	MK432641	MK578117	MK578044	MK442963	MK442988
D. schini	Brazil	*Schinus terebinthifolius*	CBS 133181*	KC343191	KC343917	KC344159	KC343433	KC343675
D. schoeni	Italy	*Schoenus nigricans*	MFLU 15-1279*	KY964226	KY964182	KY964109	KY964139	
D. sclerotioides	Netherlands	*Cucumis sativus*	CBS 296.67*	KC343193	KC343919	KC344161	KC343435	KC343677
D. searlei	Australia	*Macadamia*	CBS 146456*	MN708231	NA	MN696540	NA	NA
D. sennae	China	*Senna bicapsularis*	CFCC 51636*	KY203724	KY228885	KY228891	KY228875	NA
D. sennae	China	*Senna bicapsularis*	CFCC 51637	KY203725	KY228886	KY228892	KY228876	NA
D. serafiniae	Australia	*Helianthus annuus*	BRIP 55665a*	KJ197274	KJ197236	KJ197254	NA	NA
D. siamensis	Thailand	*Dasymaschalon* sp.	MFLUCC 10-0573a*	JQ619879	JX275393	JX275429	JX197423	NA
D. sinensis	China	*Amaranthus* sp.	ZJUP0033-4*	MK637451	MK660449	MK660447	NA	MK660451
D. sojae	USA	*Glycine max*	FAU635*	KJ590719	KJ590762	KJ610875	KJ612116	KJ659208
D. spinosa	China	*Pyrus pyrifolia*	CGMCC 3.19602*	MK626849	MK654811	MK691234	MK691129	MK726156
D. stewartii	Not stated	*Cosmos bipinnatus*	CBS 193.36*	MH867279	GQ250324	JX275421	JX197415	NA
D. subellipicola	China	On dead wood	KUMCC 17-0153*	MG746632	MG746633	MG746634	NA	NA
D. subordinaria	New Zealand	*Plantago lanceolata*	CBS 464.90*	KC343214	KC343940	KC344182	KC343456	KC343698
D. taiwanensis	Taiwan	*Ixora chinensis*	NTUCC 18-105-1*	MT241257	MT251199	MT251202	MT251196	NA
D. taoicola	China	*Prunus persica*	MFLUCC 16-0117*	KU557567	KU557635	KU557591	NA	NA
D. tarchonanthi	South Africa	*Tarchonanthus littoralis*	CBS 146073*	MT223794	NA	MT223733	NA	MT223759

Species	Location	Host	Strain	GenBank Accession Number				
				ITS	*tef1*	*tub2*	*cal*	*his3*
D. tecomae	Brazil	*Tabebuia* sp.	CBS 100547*	KC343215	KC343941	KC344183	KC343457	KC343699
D. tectonae	Thailand	*Tectona grandis*	MFLUCC 12-0777*	KU712430	KU749359	KU743977	KU749345	NA
D. tectonendophytica	Thailand	*Tectona grandis*	MFLUCC 13-0471*	KU712439	KU749367	KU743986	KU749354	NA
D. tectonigena	China	*Tectona grandis*	MFLUCC 12-0767*	KU712429	KU749371	KU743976	KU749358	NA
D. tectonigena	China	*Camellia sinensis*	LC6512	KX986782	KX999174	KX999214	KX999284	KX999254
D. terebinthifolii	Brazil	*Schinus terebinthifolius*	CBS 133180*	KC343216	KC343942	KC344184	KC343458	KC343700
D. thunbergiicola	Thailand	*Thunbergia laurifolia*	MFLUCC 12-0033*	KP715097	KP715098	NA	NA	NA
D. tulliensis	Australia	*Theobroma cacao*	BRIP 62248a*	KR936130	KR936133	KR936132	NA	NA
D. ueckeri	USA	*Cucumis melo*	FAU656*	KJ590726	KJ590747	KJ610881	KJ612122	KJ659215
D. unshiuensis	China	*Fortunella margarita*	CGMCC 3.17566*	KJ490584	KJ490463	KJ490405	NA	KJ490526
D. unshiuensis	China	*Carya illinoensis*	CFCC 52594	MH121529	MH121571	MH121606	MH121447	MH121487
D. unshiuensis	China	*Carya illinoensis*	CFCC 52595	MH121530	MH121572	MH121607	MH121448	MH121488
D. vawdreyi	Australia	*Psidium guajava*	BRIP 57887a	KR936126	KR936129	KR936128	NA	NA
D. vexans	USA	*Solanum melongena*	CBS 127.14	KC343229	KC343955	KC344197	KC343471	KC343713
D. viniferae	China	*Vitis vinifera*	JZB320071*	MK341550	MK500107	MK500112	MK500119	NA
D. vochysiae	Brazil	*Vochysia divergens*	LGMF1583*	MG976391	MK007526	MK007527	MK007528	MK033323
D. xishuangbanica	China	*Camellia sinensis*	CGMCC 3.18283*	KX986784	KX999176	KX999217	NA	NA
D. xishuangbanica	China	*Camellia sinensis*	LC6707	KX986783	KX999175	KX999216	NA	KX999255

Notes: NA, not applicable. * ex-type strains.

1000 bootstrap replicates while BI was performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.0 (Ronquist and Huelsenbeck 2003). Two MCMC chains, started from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of trees were discarded as burn-in of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP) were estimated in the remaining 7500 trees. Phylogenetic trees were visualized with FigTree v.1.3.1 (Rambaut and Drummond 2010) and processed by Adobe Illustrator CS5. The nucleotide sequence data of the new taxa were deposited in GenBank (Table 2)

Results

Phylogenetic analyses

In the present study, we inferred a genus tree of *Diaporthe* covering a large proportion of sequence data available as last summarized by Norphanphoun et al. (2022). Two strains CFCC 58154 and GZ13B formed a clade in the *D. arecae* species complex, and the other strains CFCC 58151 and GZ13E in the *D. sojae* species complex.

In the *D. arecae* species complex, the combined sequence alignments comprised 61 strains, with *D. eucalyptorum* (CBS 13252), *D. biconispora* (ZJUD62) and *D. vawdreyi* (BRIP 57887a) as the outgroup taxa. The dataset comprised 2662 characters including alignment gaps (590 for ITS, 499 for *cal*, 485 for *his3*, 375 for *tef1* and 713 for *tub2*). CFCC 58154 and GZ13B from *Bauhinia variegata* formed a distinct clade close to *D. sennae* (Fig. 1). In the *D. sojae* species complex, the combined sequence alignments comprised 166 strains (Fig. 2), with *D. aceris* (LC8112) and *D. alnea* (CBS 146.46) as the outgroup taxa. The dataset comprised 3025 characters including alignment gaps (602 for ITS, 592 for *cal*, 521 for *his3*, 483 for *tef1* and 827 for *tub2*). CFCC 58151 and GZ13E from *B. variegata* clustered in a distinct clade close to *D. tulliensis* (Fig. 2).

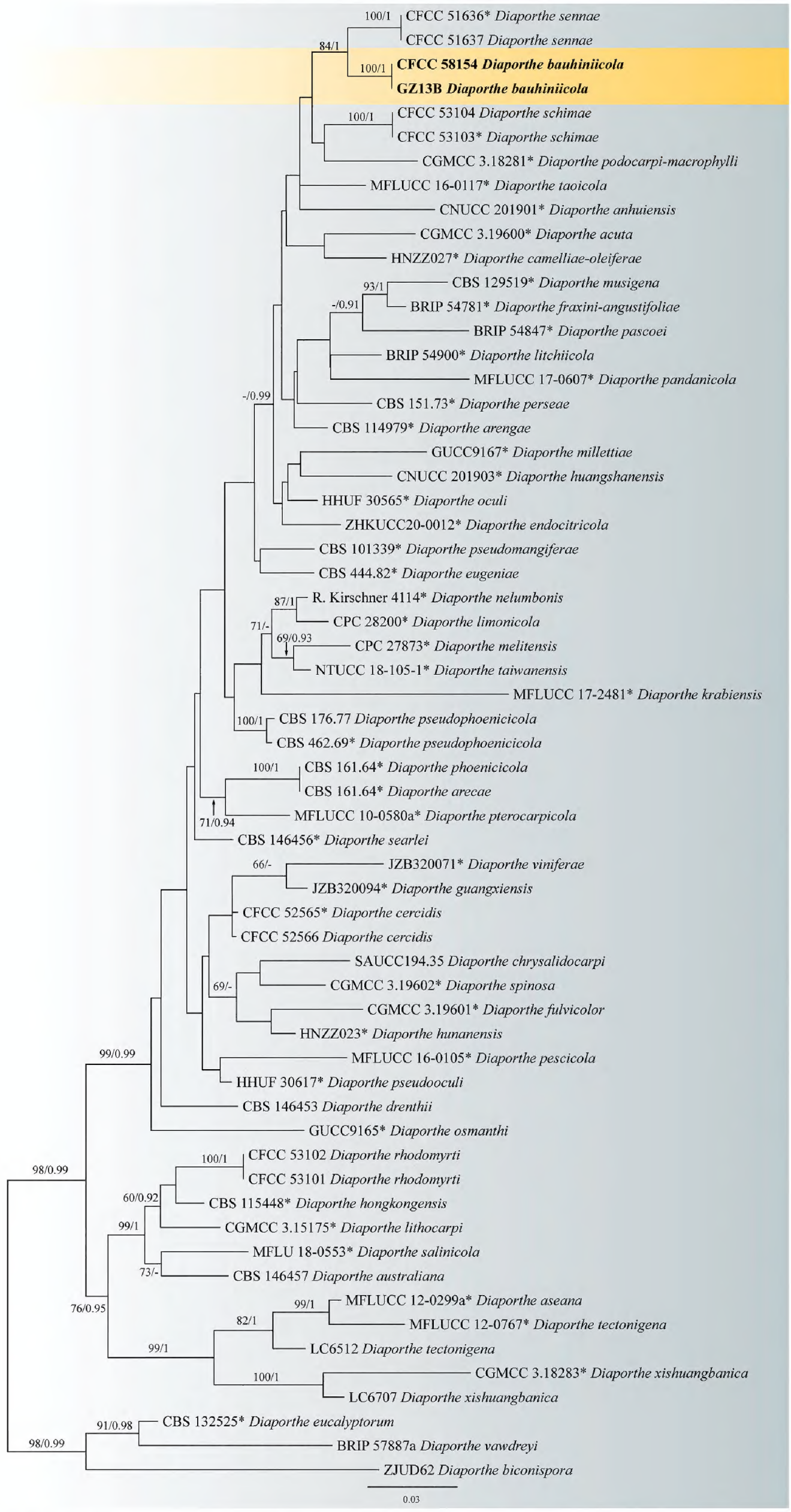

Figure1. Phylogram of *Diaporthe arecae* species complex resulting from a maximum likelihood analysis based on a combined matrix of ITS, *cal*, *his3*, *tef1* and *tub2* loci. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian posterior probabilities (right, BPP ≥ 0.9). Isolates from the present study are in bold and ex-type strains are marked with *.

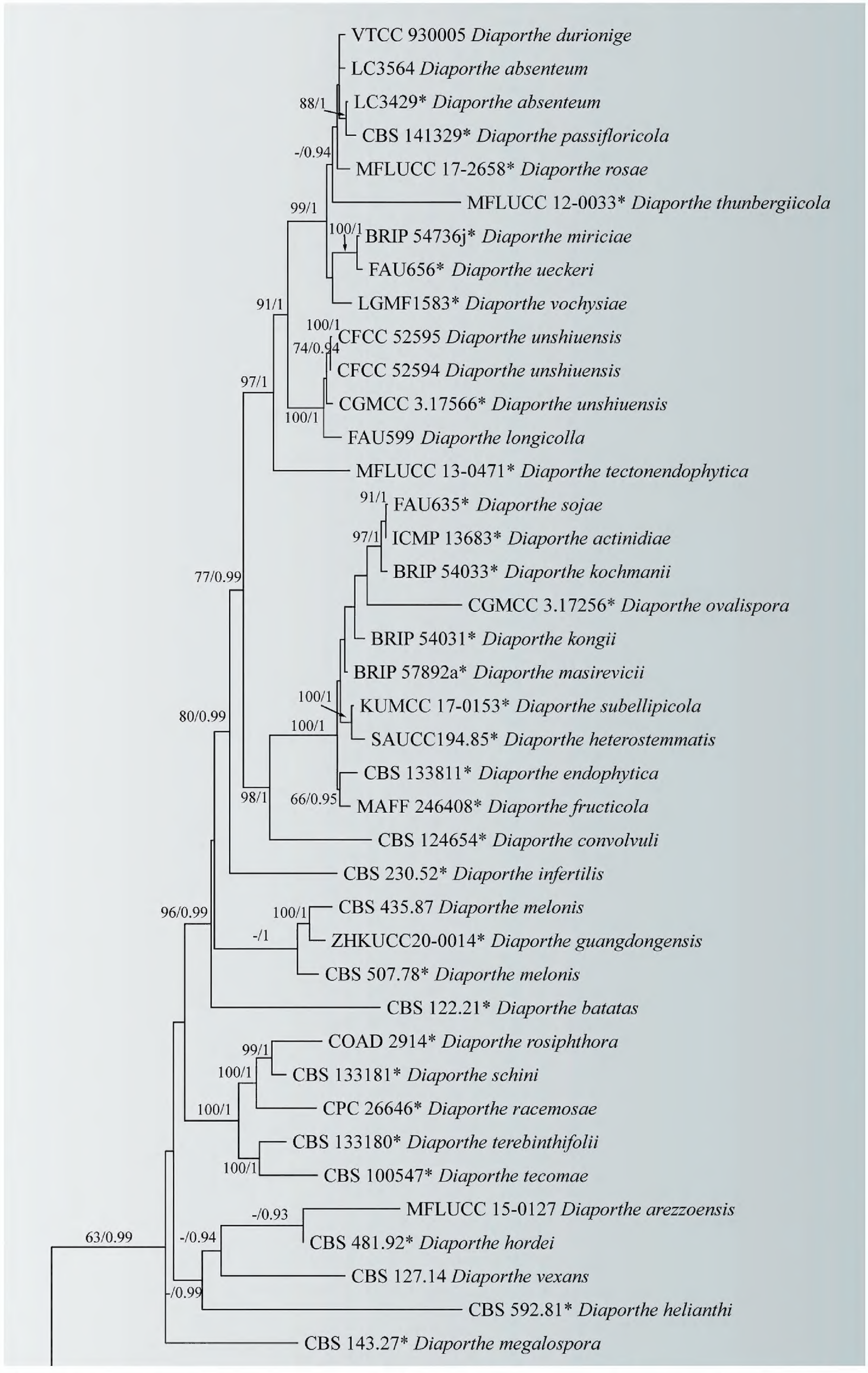

Figure 2. Phylogram of *Diaporthe sojae* species complex resulting from a maximum likelihood analysis based on a combined matrix of ITS, *cal*, *his3*, *tef1* and *tub2* loci. Numbers above the branches indicate ML bootstrap values (left, ML BS ≥ 50%) and Bayesian posterior probabilities (right, BPP ≥ 0.9). Isolates from the present study are in bold and ex-type strains are marked with *.

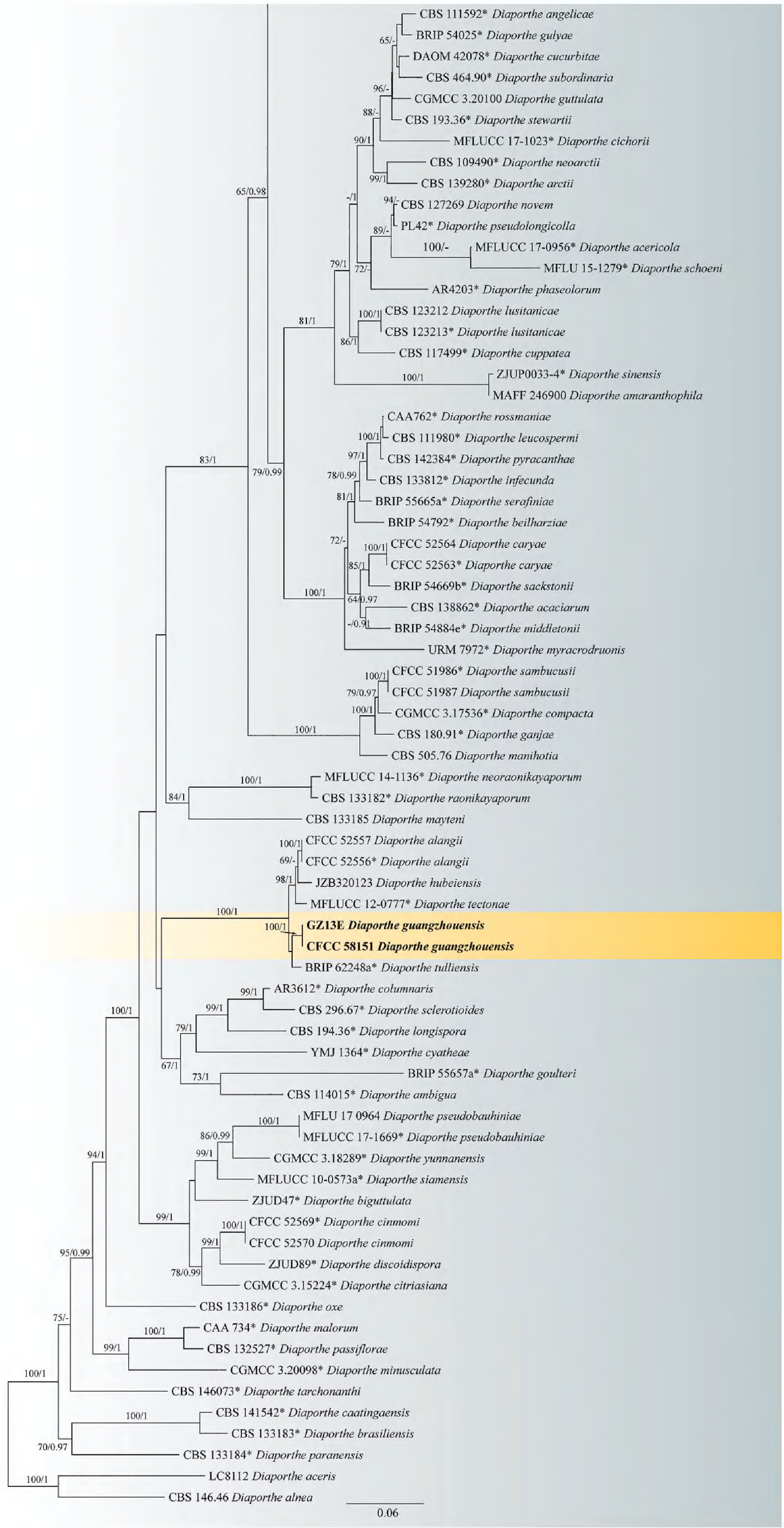

Figure 2. Continued.

Taxonomy

Diaporthe bauhiniicola **Ning Jiang & Y.Q. Zhu, sp. nov.**
MycoBank No: 854183
Fig. 3

Holotype. CHINA • Guangdong Province, Guangzhou City, Luhu Park, 23°9'11.15"N, 113°16'46.01"E, 92 m asl, on diseased leaves of *Bauhinia variegata*, 8 Aug 2022, Yong Li, Chengbin Wang & Yaquan Zhu, (holotype: CAF800094; ex-type culture: CFCC 58154).

Etymology. Named after the host genus, *Bauhinia*.

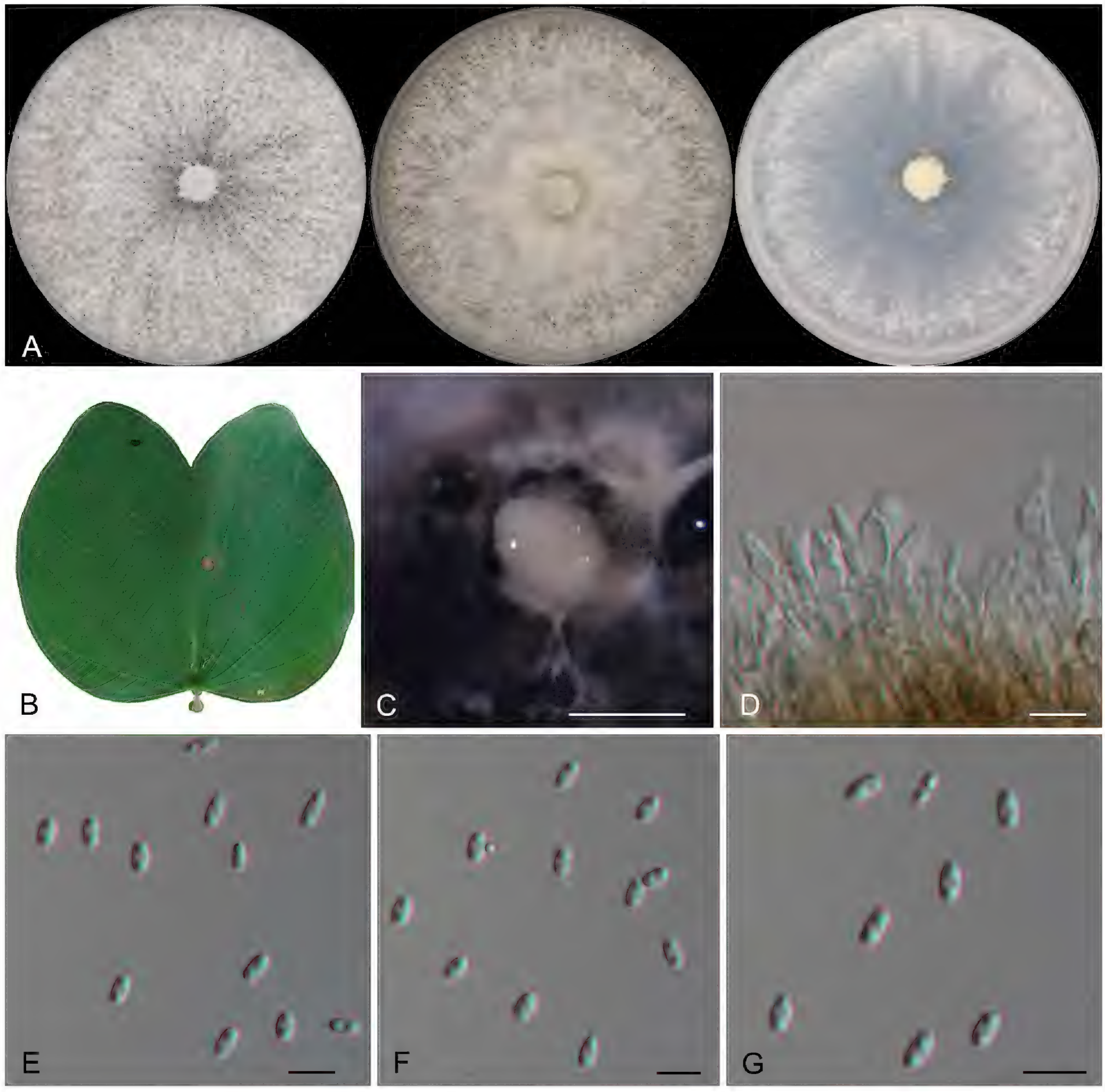

Figure 3. Morphology of *Diaporthe bauhiniicola* **A** colonies on PDA, MEA and SNA at 25 °C after 2 weeks **B** a diseased leaf of *Bauhinia variegata* **C** conidioma formed on PDA after 30 days **D** conidiogenous cells with attached alpha conidia **E–G** alpha conidia. Scale bars: 200 μm (**C**); 10 μm (**D–J**).

Description. ***Conidiomata*** formed on PDA pycnidial, scattered to aggregated, black, erumpent, raising above surface of culture medium, subglobose, 150–300 µm diam., exuding white or yellowish creamy conidial droplets from central ostioles after 30 days at 25 °C. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** hyaline, unbranched, septate, straight, slightly tapering towards the apex, 6.0–15.0 × 1.5–4.0 µm. ***Alpha conidia*** hyaline, aseptate, ellipsoidal to spindle-shaped, biguttulate or with one guttulate, 4.5–7.0 × 2.0–3.0 µm. ***Beta conidia and gamma conidia*** not observed. *Teleomorph* not observed.

Culture characteristics. Colonies covering entire plate after 2 weeks. On PDA with profuse aerial mycelium, white surface, reverse fulvous. On MEA with fluffy aerial mycelium, dirty white surface, reverse ochreous. On SNA white sparse aerial mycelium, surface and reverse white.

Additional material examined. China • Guangdong Province, Guangzhou City, Luhu Park, 23°9'11.15"N, 113°16'46.01"E, 92 m asl, on diseased leaves of *Bauhinia variegata*, 8 Aug 2022, Yong Li, Chengbin Wang & Yaquan Zhu, living culture GZ13B.

Notes. Two strains representing *Diaporthe bauhiniicola* clustered in a clade distinct from its closest phylogenetic neighbour, *D. sennae* (Fig. 1). *D. sennae* has been reported from the host *Senna bicapsularis* in China (Yang et al. 2017). *D. bauhiniicola* differs from *D. sennae* by wider alpha conidia (4.5–7.0 × 2.0–3.0 µm in *D. bauhiniicola* vs. 5.0–6.5 × 1.5–1.8 µm in *D. sennae*) (Yang et al. 2017). *Diaporthe bauhiniicola* differs from *D. sennae* in nucleotide sequence data (18/529 in ITS, 5/490 in *cal*, 15/351 in *tef1*, 14/677 in *tub2*) (Yang et al. 2017).

Diaporthe guangzhouensis **Ning Jiang & Y.Q. Zhu, sp. nov.**

MycoBank No: 854184

Fig. 4

Etymology. Named after the collection site of the type specimen, Guangzhou City.

Holotype. China • Guangdong Province, Guangzhou City, Longdong straight street, 23°11'41.02"N, 113°22'8.33"E, 46 m asl, on diseased leaves of *Bauhinia variegata*, 8 Aug 2022, Yong Li, Chengbin Wang & Yaquan Zhu, (holotype: CAF800095; ex-type culture: CFCC 58151).

Description. ***Conidiomata*** pycnidial, scattered to aggregated, black, erumpent, raising above surface of culture medium, subglobose, 150–450 µm diam, exuding white or yellowish creamy conidial droplets from central ostioles after 30 days at 25 °C. ***Conidiophores*** 12.5–24.5 × 1–2.5 µm, cylindrical, hyaline, unbranched, straight to sinuous. ***Conidiogenous cells*** densely aggregated, phialidic, unbranched, straight or slightly curved, 5.5–10 × 2.0–7.5 µm. ***Beta conidia*** filiform, hyaline, straight or slightly curved, aseptate, 17.0–29.5 × 1.0–2.0 µm. ***Alpha conidia and gamma conidia*** not observed. ***Teleomorph*** not observed.

Culture characteristics. Colonies covering entire plate after 2 weeks. On PDA with profuse aerial mycelium, white surface, reverse amber. On MEA with fluffy aerial mycelium, dirty white surface, reverse ochreous. On SNA white sparse aerial mycelium, surface and reverse white.

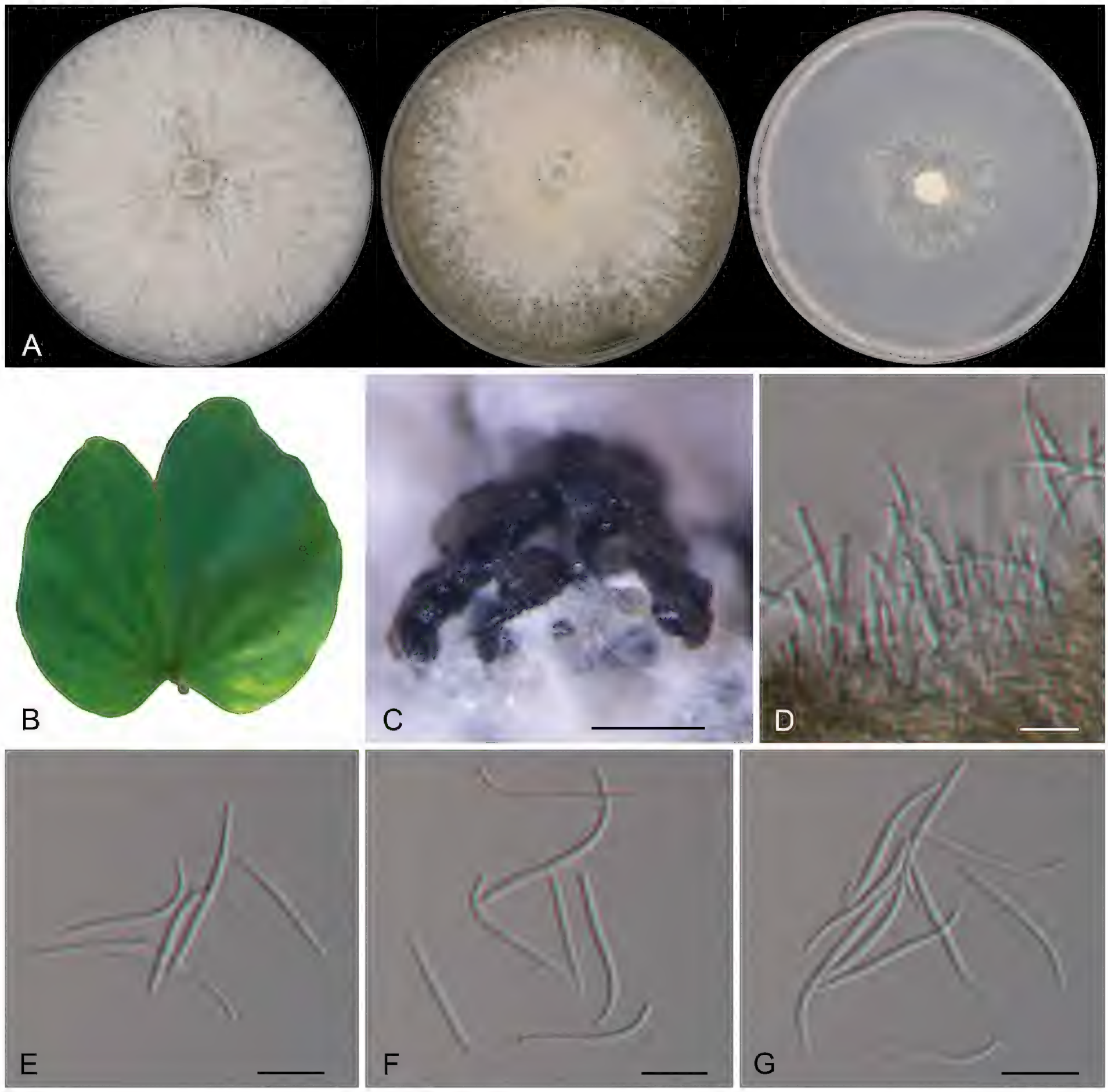

Figure 4. Morphology of *Diaporthe guangzhouensis* **A** colonies on PDA, MEA and SNA at 25 °C after 2 weeks **B** the leaf of *Bauhinia variegata* **C** conidiomata **D** conidiogenous cells with attached beta conidia **E–G** beta conidia. Scale bars: 200 µm (**C**); 10 µm (**D–J**).

Additional material examined. CHINA • Guangdong Province, Guangzhou City, Longdong straight street, 23°11'41.02"N, 113°22'8.33"E, 46 m asl, on diseased leaves of *Bauhinia variegata*, 8 Aug 2022, Yong Li, Chengbin Wang & Yaquan Zhu, living culture GZ13E.

Notes. *Diaporthe guangzhouensis* from the present study is phylogenetically close to *D. tulliensis* (Fig. 2). *Diaporthe guangzhouensis* differs from *D. tulliensis* in nucleotide sequence data (5/526 in ITS, 9/347 in *tef1*, 13/711 in *tub2*) (Crous et al. 2015). In addition, host and distribution data are vital for species identification (*D. guangzhouensis* inhabiting *Bauhinia variegata* in China vs. *D. tulliensis* inhabiting *Theobroma cacao* in Australia) (Crous et al. 2015).

Discussion

In the current study, phylogenetic analyses based on five combined loci (ITS, *cal*, *his3*, *tef1* and *tub2*), as well as morphological characters of the anamorph obtained in culture, revealed *D. bauhiniicola* and *D. guangzhouensis* spp. nov. from *Bauhinia variegata*, which contributed to our knowledge of the diversity of *Diaporthe* species in China.

Diaporthe pseudobauhiniae (syn. *D. chiangraiensis*, *Chiangraiomyces bauhiniae*) was described as a saprobic fungus on branches of *Bauhinia* sp. in Thailand (Senanayake et al. 2017). *D. bauhiniae* was introduced from branches of *B. purpurea* in China (Yang et al. 2021). Hence, a total of four species of *Diaporthe* have been recorded from the host genus *Bauhinia*. Phylogenetically, *D. bauhiniae* belongs to *D. varians* species complex; *D. bauhiniicola* belongs to *D. arecae* species complex; *D. guangzhouensis* and *D. pseudobauhiniae* belong to *D. sojae* species complex (Figs 1, 2) (Norphanphoun et al. 2022). Furthermore, *D. guangzhouensis* and *D. pseudobauhiniae* formed different clades in *D. sojae* species complex (Fig. 2). Morphologically, *D. bauhiniicola* has larger alpha conidia than *D. pseudobauhiniae*, but longer alpha conidia than *D. bauhiniae* (4.5–7.0 × 2.0–3.0 µm in *D. bauhiniicola* vs. 3–5 × 2–4 µm in *D. pseudobauhiniae* vs. 7.5–14 × 1.5–3 µm in *D. bauhiniae*) (Senanayake et al. 2017; Yang et al. 2021). *D. guangzhouensis* shares similar beta conidia size with *D. pseudobauhiniae* that are shorter and wider than *D. bauhiniae* (17.0–29.5 × 1.0–2.0 µm in *D. guangzhouensis* vs. 18–38 × 1.5–2 µm in *D. pseudobauhiniae* vs. 25–43 × 1 µm in *D. bauhiniae*) (Senanayake et al. 2017; Yang et al. 2021). Another species named *Phomopsis bauhiniae* was recorded on the branches of *Bauhinia variegata* in Spain, however, this species was only studied in morphology and has not been combined in *Diaporthe* (Uecker 1988). *Diaporthe bauhiniicola* has shorter but wider alpha conidia than *P. bauhiniae* morphologically (Uecker 1988). The molecular analyses are necessary for the species *P. bauhiniae* based on the ex-type culture in the future.

The initial species concept of *Diaporthe* based on the assumption of host-specificity, resulted in the introduction of more than 1000 taxa (http://www.indexfungorum.org/). However, more than one species of *Diaporthe* have been often discovered from the same host (Gomes et al. 2013; Guarnaccia and Crous 2017; Guarnaccia et al. 2020; Guo et al. 2020). For example, *D. caryae* and an additional 18 *Diaporthe* species are associated with pear shoot canker in China (Guo et al. 2020); *D. sennae* and *D. sennicola* inhabit branches of *Senna bicapsularis* causing canker diseases (Yang et al. 2017). The current study further supports this phenomenon.

Diaporthe is considered as a species-rich genus. Nevertheless, an emerging perspective posits that the quantity of recognized *Diaporthe* species may have been substantially overestimated. The *D. amygdali* species complex has been proven a single species evidenced from the genealogical concordance phylogenetic species recognition principle (GCPSR) and coalescence-based models: general mixed yule-coalescent (GMYC) and poisson tree processes (PTP), with several species becoming synonyms (Hilário et al. 2021b). Similarly, several species in the *D. eres* species complex such as *D. betulae* and *D. padina* were treated as synonyms (Hilário et al. 2021a). A comprehensive study is necessary to clarify species boundaries of *Diaporthe* in the future. This will help improve our understanding of the species concept within this genus.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: LM, YL, NJ. Methodology: YZ. Formal analysis: HX. Investigation: YL. Data Curation: LM, HX. Writing - Original draft: YZ. Writing - Review and Editing: NJ. Visualization: NJ.

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Data availability

All of the data that support the findings of this study are available in the main text.

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